Arbuscular mycorrhizal fungi improve biomass, photosynthesis, and water use efficiency of *Opuntia ficus-indica* (L.) Miller under different water levels

Teame G KEBEDE[1,2*], Emiru BIRHANE[1,3], Kiros-Meles AYIMUT[4], Yemane G EGZIABHER[4]

[1] Department of Land Resource Management and Environmental Protection, College of Dryland Agriculture and Natural Resource, Mekelle University, Mekelle 231, Ethiopia;
[2] Department of Animal Production and Technology, College of Agriculture and Environmental Science, Adigrat University, Adigrat 50, Ethiopia;
[3] Institute of Climate and Society, Mekelle University, Mekelle 231, Ethiopia;
[4] Department of Dryland Crop and Horticultural Science, College of Dryland Agriculture and Natural Resource, Mekelle University, Mekelle 231, Ethiopia

Abstract: *Opuntia ficus-indica* (L.) Miller is a CAM (crassulacean acid metabolism) plant with an extraordinary capacity to adapt to drought stress by its ability to fix atmospheric CO_2 at nighttime, store a significant amount of water in cladodes, and reduce root growth. Plants that grow in moisture-stress conditions with thick and less fine root hairs have a strong symbiosis with arbuscular mycorrhizal fungi (AMF) to adapt to drought stress. Water stress can limit plant growth and biomass production, which can be rehabilitated by AMF association through improved physiological performance. The objective of this study was to investigate the effects of AMF inoculations and variable soil water levels on the biomass, photosynthesis, and water use efficiency of the spiny and spineless *O. ficus-indica*. The experiment was conducted in a greenhouse with a full factorial experiment using *O. ficus-indica* type (spiny or spineless), AMF (presence or absence), and four soil water available (SWA) treatments through seven replications. Water treatments applied were 0%–25% SWA (T1), 25%–50% SWA (T2), 50%–75% SWA (T3), and 75%–100% SWA (T4). Drought stress reduced biomass and cladode growth, while AMF colonization significantly increased the biomass production with significant changes in the physiological performance of *O. ficus-indica*. AMF presence significantly increased biomass of both *O. ficus-indica* plant types through improved growth, photosynthetic water use efficiency, and photosynthesis. The presence of spines on the surface of cladodes significantly reduced the rate of photosynthesis and photosynthetic water use efficiency. Net photosynthesis, photosynthetic water use efficiency, transpiration, and stomatal conductance rate significantly decreased with increased drought stress. Under drought stress, some planted mother cladodes with the absence of AMF have not established daughter cladodes, whereas AMF-inoculated mother cladodes fully established daughter cladodes. AMF root colonization significantly increased with the decrease of SWA. AMF caused an increase in biomass production, increased tolerance to drought stress, and improved photosynthesis and water use efficiency performance of *O. ficus-indica*. The potential of *O. ficus-indica* to adapt to drought stress is controlled by the morpho-physiological performance related to AMF association.

Keywords: biomass; cactus pear; cladode growth; photosynthesis; water stress; water use efficiency

*Corresponding author: Teame G KEBEDE (E-mail: teame2004@gmail.com)

1   Introduction

Drought stress affects the performance of plants (Taghizadeh et al., 2023). It affects plant physiological and metabolic processes (Mohammadi et al., 2017; Hu et al., 2020). And this can cause function and interaction alterations of soil microorganisms (Wang et al., 2022; Qianqian et al., 2023). In arid and semi-arid areas, interactions between plants and soil microorganisms play an important role in inducing plant resistance to drought stress and in maintaining the performance of plant communities (Birhane et al., 2012; Hu et al., 2020; Taghizadeh et al., 2023). Among soil microorganisms, rhizobacteria and fungi are key actors for maintaining the productivity performance of host plants under drought stress (Wang et al., 2022; Taghizadeh et al., 2023). For instance, bacteria like Actinobacteria produce substances (Stevenson and Hallsworth, 2014), and Ascomycota fungi through decomposing complex organic matter (Chen et al., 2019) maintain plant productivity. Arbuscular mycorrhizal fungi (AMF) can also play a significant role in inducing plant resistance to drought stress and maintaining plant productivity (Hu et al., 2020; Stevens et al., 2020).

Plants benefit from water uptake by AMF hyphae, and AMF acquires carbohydrates from plants (Aiqun et al., 2017). This process helps them to form their mutual affiliation (Garcia et al., 2017). AMF can acquire up to 20% of the total carbon fixed by their host plants (Parniske, 2008). Under water-limited conditions, rhizosphere soil water available is inadequate and the roots of terrestrial plants cannot easily spread and capture water from the bulk zone (Cristina et al., 2017). Naturally, the roots become active and develop more association with AMF to enhance water uptake through AMF hyphae (Alho et al., 2015; Cristina et al., 2017).

Previous studies have examined the effect of AMF inoculation on inducing drought resistance and maintaining the productivity of plant communities. For instance, Birhane et al. (2012) studied the effect of artificial AMF inoculation on inducing drought stress and maintaining *Boswellia papyrifera* (Del.) Hochst. seedlings' productivity, and they found that AMF inoculation had a significant effect on plant resistance to drought stress and maintaining productivity. Pereira et al. (2021) investigated the effects of AMF inoculation on drought tolerance and survival of *Cenostigma microphyllum* Tul. seedlings, and they found that AMF-inoculated plants easily rehabilitate from rehydration under water stress. Hu et al. (2020) found that AMF colonized maize plants better induce to tolerance to drought stress and improve productivity than non-mycorrhizal plants. These investigations have indicated that AMF inoculation improves drought resistance and the productivity performance of host plants. However, these studies mainly focused on the effect of AMF inoculation on C_4 and C_3 plants. The impacts of AMF inoculation on different plant species mainly crassulacean acid metabolism (CAM) plants are relatively rare, and only a few studies assessed native AMF colonization on some CAM plants growing in different land use types. For instance, Pimienta-Barrios et al. (2001) found native AMF colonization of *Opuntia robusta* J.C. Wendl. improves adaptations for prolonged drought conditions. Birhane et al. (2017, 2020) observed that *O. ficus-indica* growing in arid and semi-arid areas of the Tigray region, Ethiopia was colonized by AMF. Moreover, these studies also focused on the degree of native AMF colonization in dry and rainy seasons. However, the role of artificial AMF inoculation on adaptation to drought stress of *O. ficus-indica* has not been investigated.

O. ficus-indica belongs to the genus *Opuntia*, sub-family Opuntiodeae, and family Cactaceae (Snyman, 2005; Astello-Garcia et al., 2015). It is indigenous to Central Mexico and the Caribbean region (Salem-Fnayou et al., 2014). *O. ficus-indica* is characterized by spiny or spineless type (Astello-Garcia et al., 2015). It is a CAM plant (Scalisi et al., 2015). The plant is widely cultivated in arid and semi-arid areas (Ochoa and Barbera, 2017; Berhe et al., 2022). It is considered as drought resistant, tolerant, and escaping plant (Liguori et al., 2013; Scalisi et al., 2015). Previous studies on the drought stress adaptation in this plant have mainly focused on its

ability to fix atmospheric CO_2 at nighttime (Ranjan et al., 2016), store a significant amount of water in tissue (Nobel, 2010; Scalisi et al., 2015), and transfer water from water-storage tissue (parenchyma) to photosynthetic tissue (chlorenchyma) (Andrade et al., 2009). The morphological traits such as the presence of spines, areoles, and cladode surface area can be related to the drought stress tolerance of *O. ficus-indica* (Pena-Valdivia et al., 2007; Ranjan et al., 2016). These investigations showed that drought stress adaptation of the plant was related to the nature and phenomena of the cladode. However, drought stress adaptation of the plant related to the root is rare. Snyman (2004, 2005) investigated the relation of drought stress to the root dynamics of *O. ficus-indica*, and found that the root biomass, thickness, length, and the number of fine roots of the plant decrease significantly with an increase in drought stress. However, these studies did not assess the relationship between AMF root colonization and drought stress adaptation of *O. ficus-indica*. Under drought stress conditions, AMF root colonization of plants can relate to the root dynamic of plants (Brundrett et al., 1996; Birhane et al., 2012). *O. robusta*, for example, has higher root thickness, length, and fine root than *O. ficus-indica* (Snyman, 2005), and AMF colonization increases under water-limited conditions (Pimienta-Barrios et al., 2001). These investigations may indicate that AMF could be of more benefit under drought stress because plants with short, thick, and less fine root hairs benefit better, and AMF can replace the fine root hairs and serve in the absorption and transport of minerals and water from the soil micro-pores (Brundrett et al., 1996). AMF improves the morphological performance mainly biomass production of host plants by facilitating increased growth, root collar diameter, root length, number of leaves, and leaf area (Birhane et al., 2012; Andrino et al., 2020; Pereira et al., 2021). It also helps to improve physiological performance by increasing photosynthesis rate and water use efficiency (Birhane et al., 2012; Andrino et al., 2020; Pereira et al., 2021). Increases in morpho-physiological performance are ensured by the combinations of nutritional, morphological, and physiological (Birhane et al., 2012), nutritional and non-nutritional (Auge et al., 2016), and physiological and metabolic mechanisms (Hu et al., 2020). These microbes are also significantly important in increasing photosynthetic water use efficiency through decreased transpiration rates (Birhane et al., 2012; Pereira et al., 2021). AMF colonization also increases the morpho-physiological performance of plants through improved soil physical and chemical properties (Hailemariam et al., 2017). However, AMF abundance (spore density) in soil can decrease with an increase in drought stress and soil phosphorus availability (Stevens et al., 2020). Its function is also affected by soil disturbance (Trejo et al., 2016).

O. ficus-indica growing in the Tigray region, Ethiopia is traditionally cultivated without irrigation and inorganic fertilizers (Belay et al., 2011). The plant is also dominated by the spiny type (Ochoa and Barbera, 2017). In arid and semi-arid areas, the frequent occurrence of drought and growing more spiny type are major plant performance constraints as they have a negative effect on growth and biomass production of *O. ficus-indica* (Snyman, 2005; Belay et al., 2011; Scalisi et al., 2015), photosynthesis rate (Pimienta-Barrios et al., 2005; Liguori et al., 2013), and water use efficiency (Snyman, 2005). Thus, improvement of plant accessions and an increase in plant soil water available can improve the morpho-physiological performance of *O. ficus-indica* cladodes (Snyman, 2005; Scalisi et al., 2015). However, the interactive effect of plant soil water available and *O. ficus-indica* type on the morpho-physiological performance of cladodes to AMF inoculation is unclear. In this study, we focused on the role of AMF symbiosis on the performance of spiny and spineless *O. ficus-indica* under various soil water available (SWA) treatments. It also reported how the symbiosis of the AMF affected the productivity performance of *O. ficus-indica*. We hypothesized that: (1) spiny *O. ficus-indica* cladodes show lower morpho-physiological performance than spineless; (2) mycorrhizal *O. ficus-indica* cladodes show higher morpho-physiological performance than cladodes without AMF; (3) morpho-physiological performance of *O. ficus-indica* cladodes increases with increase in soil water available; (4) mycorrhizal spineless *O. ficus-indica* cladode types show higher morpho-physiological performance than mycorrhizal spiny cladodes under less water levels; and (5) AMF improve the performance of both spineless and spiny *O. ficus-indica* cladodes with decrease in plant soil water available.

2   Materials and methods

The experiment was conducted at the greenhouse of Mekelle research center in the Mekelle City, Tigray, Ethiopia (13°29′N, 39°28′E; 2000 m a. s. l.) from September 2019 to March 2021. The greenhouse had mean day/night temperatures of 26°C/22°C. Mean daily average relative humidity of the greenhouse was 51%.

2.1   Mother cladodes and soil preparation

One-year-old spiny and spineless mother cladodes of green *O. ficus-indica* were obtained from Mekelle research center. We selected mother cladodes based on their morphological traits following Snyman (2005) and Belay et al. (2011) procedures. Cladodes used for the study were of similar weight, size, and thickness, and number of areoles per pad differed only in the presence or absence of spines (Table 1).

Table 1   Mean fresh cladode weight, height, breadth, thickness, number of areoles, and spines per cladode of *O. ficus-indica* mother plants

Feature	Unit	Mother cladode type	
		F	*P*
Fresh weight	g	0.106	0.285
Height	cm	0.047	0.860
Breadth	cm	0.017	0.355
Thickness	cm	2.704	0.256
Areoles per cladode	number	8.666	0.203
Spines per cladode	number	73.299	<0.001

The cladodes were green, healthy, and fresh. The spiny cladodes were 35.58 (±0.21), 19.53 (±0.16), and 1.09 (±0.54) cm long, wide, and thick, respectively (Table 1). The number of areoles and spines per cladode were 90,020.35 and 270.05, respectively, with the mean fresh weight of 968.00 (±0.33) g. Spineless candidates were 35.58 (±0.21) cm long, 19.55 (±0.16) cm wide, 1.08 (±1.00) cm thick, 969.48 (±0.33) g fresh weight, and with a total number of 89.36 (±0.25) areoles. After collection, cladodes were air-dried at room temperature under shelter and shaded for four weeks (Snyman, 2004). The samples were preserved with a mean daily relative humidity of 51% and with temperatures 25°C–30°C during the day, and 15°C–18°C at night. This helps to promote the healing of the cut area of the planting materials for successful root development (Snyman, 2005).

The soil mix used for the study was composed of three parts of soil excavated from the rhizosphere of *O. ficus-indica* plantation in the field and two parts of pure river sand. Mixed soil prepared for all pots was of uniform character. The clay, silt, and sand contents of the soil were 12.8%, 7.1%, and 80.1%, respectively. The soil mix had a bulk density of 1.52 g/cm³, hydrogen potential of 7.77, and electrical conductivity of 0.11 dS/m. Organic carbon, total nitrogen, available potassium, and available phosphorous contents of the soil were 1.04%, 0.7%, 0.5 mg/g, and 1.103 mg/g, respectively.

2.2   AMF extraction, cultivation, and application

Soil samples were excavated using a handheld hoe and were collected from the rhizosphere of *O. ficus-indica* growing areas following Birhane et al. (2017). For spore extraction, sample soils were first air dried and passed through a 0.75-mm sieve to remove unnecessary materials, and were weighed using a sensitive balance to get a sub-sample of 25 g mix (Brundrett et al., 1996). The weighed sample was soaked in 100 mL tap water for at least 30 min and shaken for 30 min, then was decanted through a series of sieves by putting in ascending order with the smallest sieve size (50 µm) at the bottom, followed by 100, 300, and 750 µm largest sieve size at the uppermost.

Spores on the three different sieve sizes were poured into their respective jars. Jars were well fixed and were then centrifuged at 2000 r/min for 5 min with water and poured into the smallest sieve size, and the pellet was discarded. Sieves were washed for the second time and poured into a jar filled with 50% sucrose, centrifuged for 1 min at 2000 r/min, poured onto the finest sieve (50 μm), and carefully washed with water to remove the sucrose. The pellet was discarded. Finally, spores were washed and poured onto a pre-wetted filter paper in a funnel and put in an inverted petri dish to store the filter papers with spores. Finally, spores were counted using a dissecting microscope with ×400 magnification. Then the sterile soil was mixed with Glomus, Acaulospora, and Scutellospora AMF genera types, with an average spore density of 196.6 spores/100 g dry soil. Pots were filled with 10.5 kg of soil each and AMF pot cultures were grown in a greenhouse using *Sorghum bicolor* (Linn.) Moench plants following the method of Birhane et al. (2012). The viability of the sorghum seeds was determined before planting. Twelve sorghum seeds were planted in each pot and grown for 60 d. Soil and root samples were collected from each pot culture for further determination of AMF root colonization and spore density. Spores density and AMF root colonization were determined using a compound microscope with ×400 magnification. The average spore density and AMF root colonization 60 d after planting *S. bicolor* were 198.8 spores/100 g soil and 99.21%, respectively, and these values were used to determine the weight of AMF inoculum added to the pot. The numbers of spores added to the center of each pot were close to 400 spores (198.8 spore×201.0 g/100 g soil). Fungal inoculums were composed of a mixture of soil, spores, and root fragments, and 201.0 g of inoculum was added to the center of each pot of the mother cladode. To mimic the optimum rhizosphere ecosystem and increase AMF performance, we added 300 mL microbial wash created through the extraneous extraction solution (without spores) from fungi inoculums to the center of each pot. Adding 400 numbers of spores and 300 mL microbial wash is recommended for improved performance of plants and positive protection of insects (Frew et al., 2017).

2.3 Planting cladodes

Mother cladodes of *O. ficus-indica* were planted in soil-filled cylindrical plastic containers having top and bottom diameters of 32 and 27 cm, respectively, and 30 cm in height. The cladodes were planted upright with one-quarter of their height covered with soil and were grown for 18 months. Each pot was filled with 18.50 kg weight of autoclaved and dried mix soil. Experimental plants were grown individually in the filled autoclaved growing media. The pots were having 5 drainage holes of 6.5 mm diameter at the bottom. The pots were put on their covers to save nutrients and water loss through the holes (Snyman, 2004).

2.4 Experimental design and treatment

The treatments were composed of three factors, i.e., AMF (present or absent), *O. ficus-indica* type (spiny or spineless), and four water treatments (0%–25% SWA (T1), 25%–50% SWA (T2), 50%–75% SWA (T3), and 75%–100% SWA (T4)) (Snyman, 2004). Each pot contained one cladode, and each mother cladode was replicated seven times over the treatments, making a total of 112 mother cladodes (2 *O. ficus-indica* type×2 AMF×4 SWA×7 replications) in 112 pots. In this experiment, additional 12 experimental pots filled with the same quantity of autoclaved mix soil (18.50 kg each) were considered as control and were used to determine the percent of SWA. The weight values were determined as the permanent wilting point (PWP) of the soil pots. The controlled soil pots were saturated with water and reweighed after 48 h, and the saturated soil weight of the pots was 20.64 kg, and the value was used to determine pot water capacity (PWC).

The amount of water added to every plant was determined following Snyman (2004).

$$\text{Plant soil water available=Pot water capacity–Permanent wilting point,} \quad (1)$$

$$\text{Volumetric soil water=Surface area (pot)×Height (water depth).} \quad (2)$$

Accordingly, SWA values at PWP and PWC were 0.073 and 0.285 mm water/mm soil pot depth, respectively. The total SWA was 0.212 mm water/mm soil pot depth or 63.600 mm water/pot. This value was used to determine and monitor SWA of exact water treatment.

2.5 Mycorrhizal colonization

Root samples were collected from treated cladodes and preserved in plastic jars filled with 50% ethanol (Birhane et al., 2012). Collected roots were cut into 1 cm and bleached using 10% potassium hydroxide in a heat-resistant jar. The roots were autoclaved at 121°C for 15 min (Brundrett et al., 1996). The roots were immersed in 10% hydrogen peroxide for about 15 min for further bleaching and clearing, and acidified with 2% HCl for about 1 h at room temperature. They were stained in trypan blue (0.05% in 5:1:1 lactic acid:glycerol:distilled water ratio) overnight. Stained roots were washed, immersed in 50% glycerol for 1–2 h, de-stained, and preserved until further processing. Afterwards, stained roots were mounted lengthwise on slides in replicates of nine from every individual of treated cladodes. Proportional roots colonization by AMF were estimated using the magnified intersection method with hair line graticule inserted into an eyepiece that acted as the lines of intersection with 0, 30, 60 and 90 inches following Giovannetti and Mosse (1980). AMF structures (arbuscules, vesicles, and hyphae) were identified using a compound microscope at ×400 magnification.

2.6 Measurement of morpho-physiological cladodes traits

Morpho-physiological trait measurements were done at the end of the experiment. Morphological traits measured in this experiment were the biomass of cladodes, number of daughter cladodes, number of spines, and areoles. The total number of 1st and 2nd emerging and up-growing daughter cladodes per mother cladodes, and number of spines per areole, number of areoles per cladode were manually counted and recorded. Cladode height and breadth were measured using a ruler. Thickness of the cladodes was measured using a digital caliper. Fresh plant biomass per pot was measured using a digital balance. The cladode area was calculated using the method of Tiznado-Hernández et al. (2010).

$$\text{Cladode area} = \pi \times \left(\frac{\text{Height}}{2} \right) \times \left(\frac{\text{Breadth}}{2} \right). \tag{3}$$

The height of cladodes was recorded 2 times per month and continuously measured for the first four months. It was used to evaluate the rate of growth (Scalisi et al., 2015).

Net photosynthesis rate (P_n), transpiration rate (E), and stomatal conductance (C) were measured as physiological traits of the daughter cladodes. These traits were measured using a portable CI-340 Handheld (CID Bio-Science, Inc., Camas, USA) photosynthesis system. Photosynthetic water use efficiency (PWUE) was determined following Birhane et al. (2012). The traits were also measured after 18 months of growth under the treatments. The traits were taken six times over 2400 h period with 400 h intervals on 4 March, 2021.

2.7 Statistical analysis

Data for the number of 1st emerged cladodes, biomass, height, breadth, and thickness of daughter cladodes, number of spines per areole, areoles per cladode, the number of up-growing daughter cladodes, P_n, E, C, and PWUE were subjected to analyses of variance (ANOVA) using SPSS 2004 software. Variations of AMF in *O. ficus-indica* daughter cladodes traits were tested using three-way ANOVA. Fractional root colonization of AMF in plant roots was tested using two-way ANOVA. After checking for the normality of the distribution of the data, we performed Gabriel post hoc test for unequal sample size and least significant difference (LSD) for the main effect comparison. Standard errors were used to show an estimate of variability. LSD (α=0.05) was used to compare means. *P*-values were used to show significance levels.

3 Results

3.1 Morphological traits of daughter cladodes

After nurturing the mother cladodes in the greenhouse for 18 months, we measured and analyzed the main effects of *O. ficus-indica* type, AMF, water treatment, and their interaction for the

morphological traits of the daughter cladodes (Fig. 1; Table 2). Numbers of established and up-growing daughter cladodes were not significantly affected by *O. ficus-indica* type and AMF inoculation. Fresh biomass was significantly affected by treatments. The presence of spines significantly reduced the height, breadth, and surface area of the cladodes, but improved the number of areoles per cladode (Table 2). AMF inoculation significantly increased the height, breadth, thickness, and surface areas of the cladodes. Water treatments had a significant source of variations for numbers of established, up-growing, height, breadth, thickness, and surface area of cladodes, and were significantly decreased with increasing water stress (Table 2). Significantly lower numbers of areoles were recorded in highly water-stressed cladodes. Significant interactions were observed for *O. ficus-indica* type×AMF and *O. ficus-indica* type×SWA for up-growing, cladode biomass, height, breadth, number of areoles, and total spines cladode. *O. ficus-indica* type×AMF×SWA interaction significantly affected up-growing, cladode biomass, height, breadth, thickness, number of areoles, spines areoles, and total spines cladode.

3.2 Cladode growth

Spineless daughter cladodes showed significantly higher rates of growth in 30 and 45 d of growing periods (Table 2). AMF was not a significant source of variation. Cladodes growth rates were significantly affected by water treatments, which increased growth with increasing SWA (Table 2). Cladodes growth rates were significantly affected by the interactions of *O. ficus-indica* type×AMF, *O. ficus-indica* type×SWA, AMF×SWA, and *O. ficus-indica* type×AMF×SWA. Cladodes exhibited the highest rates of growth in 60 d of growing period followed by 75 and 45 d of growing periods. Growth rates of the cladodes had similar rates in 75 d of growing period (Fig. 1).

3.3 AMF root colonization

AMF root colonization was observed only in the roots of *O. ficus-indica* inoculated with AMF. AMF root colonization was significantly affected by SWA, but the presence of spines was not significant source of variation (Table 3). Mycorrhizal colonization significantly varied by the interaction effect of *O. ficus-indica* type×SWA. The highest average fractional colonization was recorded for hyphal colonization followed by arbuscular and vesicular colonization. Hyphal, arbuscular, and vesicular colonization significantly decreased with increasing levels of SWA (Table 3).

3.4 Physiological traits of daughter cladodes

Photosynthesis rate and water use efficiency were significantly affected by *O. ficus-indica* type, AMF, and SWA (Table 2). Transpiration rate was not significantly affected by *O. ficus-indica* type and AMF, but SWA was a significant source of variation. AMF and SWA were significant sources of variations in stomatal conductance. The presence of spines in the cladodes significantly
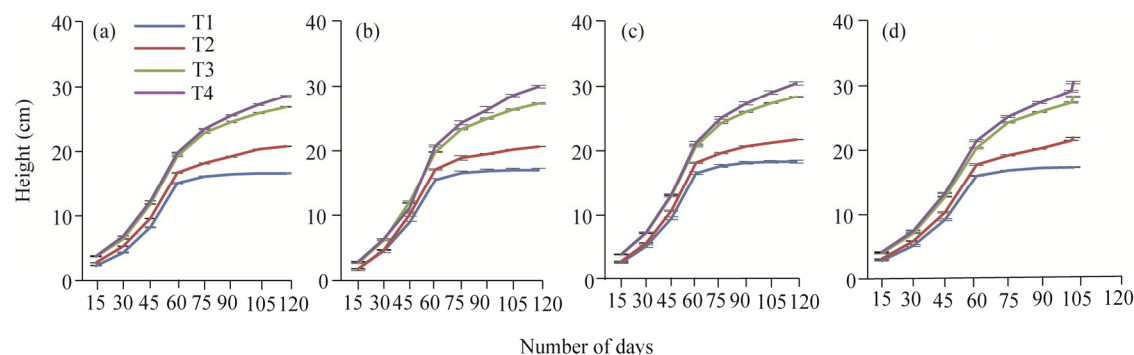

Fig. 1 Effects of *O. ficus-indica* type (spiny (a) or spineless (b)), arbuscular mycorrhizal fungi (absent (c) or present (d)), and soil water available (SWA) on the height of *O. ficus-indica* cladodes after 18 months of growth in the greenhouse. T1, 0%–25% SWA; T2, 25%–50% SWA; T3, 50%–75% SWA; T4, 75%–100% SWA. Bars are standard errors.

Table 2 Effects of *O. ficus-indica* type, arbuscular mycorrhizal fungi (AMF), soil water available (SWA), and their interaction on the morphological traits of daughter cladodes (n=108)

Parameter	Type		AMF		SWA		Type×AMF		Type×SWA		AMF×SWA		Type×AMF×SWA	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Establish cladodes	0.572	0.451	0.006	0.936	3.235	0.025	0.507	0.478	0.264	0.768	0.508	0.603	0.244	0.912
Up-growing cladodes	0.089	0.766	0.064	0.801	103.553	0.000	18.262	0.000	21.940	0.000	18.291	0.000	8.192	0.000
Height	16.419	0.000	7.274	0.008	92.286	0.000	567.007	0.000	134.662	0.000	201.308	0.000	193.694	0.000
Breadth	27.280	0.000	9.937	0.002	31.964	0.000	29.045	0.000	7.117	0.001	55.666	0.000	118.953	0.000
Thickness	0.006	0.941	18.539	0.000	63.724	0.000	0.889	0.348	2.638	0.077	6.983	0.001	2.491	0.048
Areoles	9.107	0.003	3.034	0.084	13.333	0.000	0.030	0.863	3.079	0.051	13.757	0.000	5.655	0.000
Spines areole	1445.969	0.000	1.012	0.317	0.623	0.602	13.667	0.000	14.218	0.000	71.583	0.000	51.853	0.000
Total spine cladodes	864.609	0.000	1.764	0.187	1.064	0.368	5.858	0.017	13.316	0.000	33.127	0.000	19.709	0.000
Area	20.550	0.000	9.383	0.003	79.637	0.000	0.037	0.848	0.116	0.734	0.970	0.327	0.080	0.779
Biomass	4.286	0.041	6.737	0.011	283.564	0.000	583.038	0.000	641.902	0.000	596.762	0.000	243.113	0.000
Growth rate at 15 d	3.012	0.086	0.880	0.350	230.445	0.000	69.751	0.000	51.694	0.000	71.937	0.000	29.878	0.000
Growth rate at 30 d	11.700	0.001	1.666	0.200	172.523	0.000	303.051	0.000	166.322	0.000	139.648	0.000	112.233	0.000
Growth rate at 45 d	6.447	0.013	1.516	0.221	332.090	0.000	323.051	0.000	214.124	0.000	223.398	0.000	118.398	0.000
Growth rate at 60 d	3.735	0.056	0.941	0.334	432.003	0.000	64.105	0.000	65.707	0.000	37.848	0.000	25.453	0.000
Growth rate at 75 d	1.043	0.310	0.447	0.505	1091.426	0.000	278.154	0.000	271.614	0.000	185.975	0.000	98.690	0.000
Growth rate at 90 d	0.634	0.428	0.355	0.553	1484.897	0.000	318.049	0.000	328.463	0.000	236.695	0.000	122.449	0.000
Growth rate at 105 d	0.309	0.579	0.043	0.835	2350.277	0.000	423.336	0.000	418.416	0.000	305.089	0.000	147.558	0.000
Growth rate at 120 d	0.400	0.641	0.041	0.840	3000.697	0.000	560.374	0.000	541.501	0.000	414.367	0.000	195.538	0.000
Photosynthesis at day time	5.333	0.020	1.877	0.174	358.918	0.000	2.074	0.153	0.548	0.461	0.011	0.915	0.009	0.926
Photosynthesis at night time	10.303	0.002	9.872	0.002	147.497	0.000	0.364	0.548	0.046	0.830	0.892	0.347	3.298	0.072
Transpiration at day time	0.046	0.831	0.380	0.539	1444.785	0.000	0.203	0.654	1.698	0.196	0.182	0.671	0.128	0.721
Transpiration at night time	0.707	0.402	0.474	0.493	192.021	0.000	0.132	0.717	0.124	0.726	0.078	0.780	0.408	0.524
Stomatal conductance at day time	4.438	0.038	1.017	0.316	534.654	0.000	0.339	0.562	1.635	0.204	0.000	0.993	0.136	0.713
Stomatal conductance at night time	3.726	0.056	0.390	0.539	798.901	0.000	0.973	0.326	0.001	0.977	0.540	0.464	0.218	0.642
Water use efficiency at day time	6.700	0.011	4.054	0.047	97.201	0.000	1.121	0.292	9.660	0.002	8.930	0.004	1.996	0.161
Water use efficiency at night time	14.203	0.000	13.829	0.000	100.683	0.000	1.167	0.283	0.496	0.483	1.091	0.161	1.797	0.183

Table 3 Effects of *O. ficus-indica* type, soil water available (SWA), and their interaction on the root colonization

Factor	Treatment	Hyphal	Arbuscular	Vesicular
Type	Spiny (%)	66.67±1.00[a]	32.74±0.56[a]	29.93±0.63[a]
	Spineless (%)	66.67±0.98[a]	33.94±0.60[a]	30.63±0.64[a]
	F	0.000	0.355	0.306
	P	1.000	0.671	0.806
SWA	T1 (%)	88.89±0.00[a]	45.37±0.45[a]	41.33±0.52[a]
	T2 (%)	77.78±0.00[b]	40.87±0.36[b]	39.10±0.29[b]
	T3 (%)	55.56±0.00[c]	27.25±0.27[c]	24.54±0.23[c]
	T4 (%)	44.44±0.00[d]	19.88±0.21[d]	16.18±0.13[d]
	F	5.341	1207.443	1346.818
	P	0.000	0.000	0.000
Type×SWA	*F*	30.333	22.257	22.347
	P	0.000	0.000	0.000

Note: Different lowercase letters within the same treatment indicate significant differences at *P*<0.05 level. Mean±SD.

reduced the rate of photosynthesis and photosynthetic water use efficiency throughout day and night times (Table 2). Stomatal conductance was significantly reduced during day time. At nigh time, AMF cladodes had shown significantly higher photosynthesis rates than plants without AMF. Water use efficiency was significantly higher for AMF cladodes than for control during both day and night times. Photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency decreased with increasing water stress during both day and night times (Table 2). Significantly lower physiological traits were recorded in 0%–25% level of SWA. Except for water use efficiency, all traits were not significantly affected by the interactions (Table 2).

4 Discussion

The hypothesis that spiny cladodes show lower morpho-physiological performance than spineless is supported in this study. The presence of spines significantly influenced the morphological traits mainly fresh biomass and growth rates in 30 and 45 d of growing periods (Fig. 1; Table 2). The negative spines effect is due to reduced height, breadth, and surface area of the cladodes, which were evident by the significantly lower height, breadth, and area of spiny cladodes than spineless. According to Pea-Valdivia et al. (2008), spines in *O. ficus-indica* significantly influenced the breadth and area of the cladodes. In contrast, Adli et al. (2016) reported wide and large areas of the cladodes did not significantly vary between the spiny and spineless accessions. The presence of spines also significantly affected the photosynthetic rate and photosynthetic water use efficiency of the cladodes both during the day and night times (Table 2). The decrease in P_n and PWUE in spiny cladodes was due to the presence of spines. The spines in spiny cladodes were distributed throughout the surface area of the cladodes. Spines dispensed in the surface area of the cladodes can interrupt solar energy that functions to reduce surface temperature and water loss of the cladodes (Ranjan et al., 2016). Due to different P_n and PWUE of the cladodes of the two accessions, spineless *O. ficus-indica* had high fresh biomass than spiny ones. Biomass of the cladodes increased with an increase in P_n (Scalisi et al., 2015) and water use efficiency (Snyman, 2013).

The hypothesis that cladodes with the presence of AMF show higher biomass performance than cladodes without AMF is supported in this study. Several previous studies have reported on AMF-inoculation improves the biomass production of host plants by facilitating increased growth, root collar diameter, root length, number of leaves, and leaf area (Birhane et al., 2012; Hailemariam et al., 2017; Andrino et al., 2020). These microbes are also significantly important in increasing biomass production through decreased transpiration rates (Birhane et al., 2012). In

contrast, Pereira et al. (2021) observed biomass production of plants growing at high soil water available did not significantly vary between AMF-inoculated and non-inoculated plants. Our findings suggest the presence of AMF significantly improved the height, breadth, and thickness, and the area of daughter cladodes, in turn, improved the biomass of *O. ficus-indica* (Table 2; Fig. 2). Biomass of cladodes increased with an increase in thickness (Scalisi et al., 2015). Daughter cladodes growth rates were not improved by AMF alone but were clearly increased when AMF was associated with *O. ficus-indica* type and SWA. These results are consistent with the findings of Hailemariam et al. (2017) who investigated the effects of different levels of SWA and AMF-inoculation on growth of *Faidherbia albida* (Del.) A. Chev. Specifically, the result of this study showed that at less level of SWA higher growth was observed for AMF-inoculated *F. albida* than non-mycorrhizal plants. Growth of cladodes could not affect by a single environmental factor, but also by many other factors (Scalisi et al., 2015). At less soil water availability, the root of *O. robusta* associated with AMF positively influences the establishment of new cladodes (Pimienta-Barrios et al., 2001). Similarly, Pereira et al. (2021) observed that at low plant soil water available AMF-inoculation significantly affects the growth and survival of *Cenostigma microphyllum* (Mart. ex G. Don) E. Gagnon and G. P. Lewis. Developing new daughter cladodes could improve the growth of mother cladodes (Scalisi et al., 2015). Cladodes with AMF
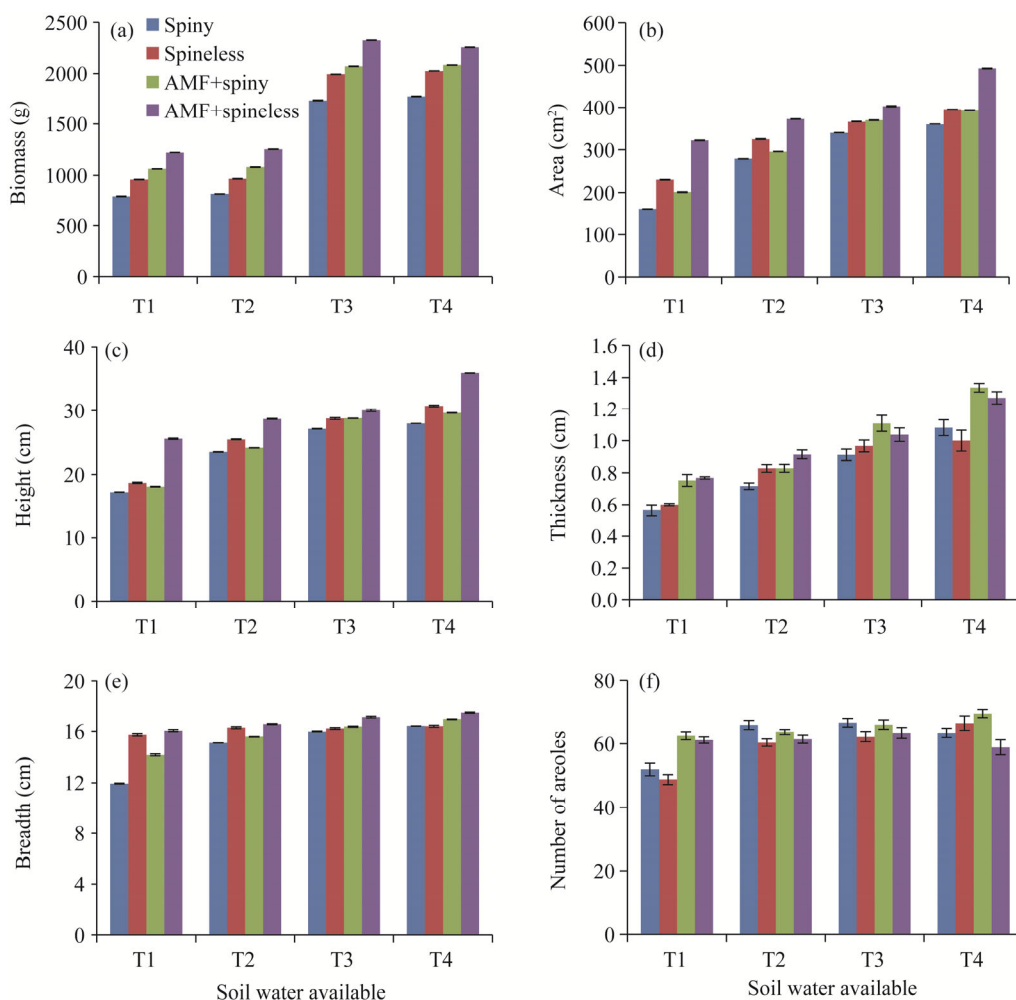
Fig. 2 Effect of interaction of *O. ficus-indica* type (spiny or spineless), arbuscular mycorrhizal fungi (AMF; absent or present), and soil water available (SWA) on biomass (a), area (b), height (c), thickness (d), breadth (e), and number of areoles (f) of *O. ficus-indica* cladodes after 18 months of growth in the greenhouse. T1, 0%–25% SWA; T2, 25%–50% SWA; T3, 50%–75% SWA; T4, 75%–100% SWA. Bars are standard errors.

improved P_n and PWUE (Table 2; Fig. 3). This was similarly reported by Birhane et al. (2012) with species of *Boswellia papyrifera* (Del.) Hochst. According to Birhane et al. (2012), *B. papyrifera* seedlings with AMF significantly influenced P_n and PWUE. Andrino et al. (2020) observed that AMF inoculation increased the leaf area of *Solanum lycopersicum* L. species, which positively influenced P_n.

We found that the morpho-physiological cladodes were more beneficiaries at less level of SWA (Table 2; Fig. 3). Increasing level of SWA can improve root biomass production of *O. ficus-indica* (Snyman, 2004, 2005). It also improves the cladode relative water content, cladode thickness, and rhyzosphere soil moisture content of *O. ficus-indica* (Scalisi et al., 2015). Thus, improving the biomass production of mycorrhizal *O. ficus-indica* plant was ensured by the combinations of morpho-physiological performance change.
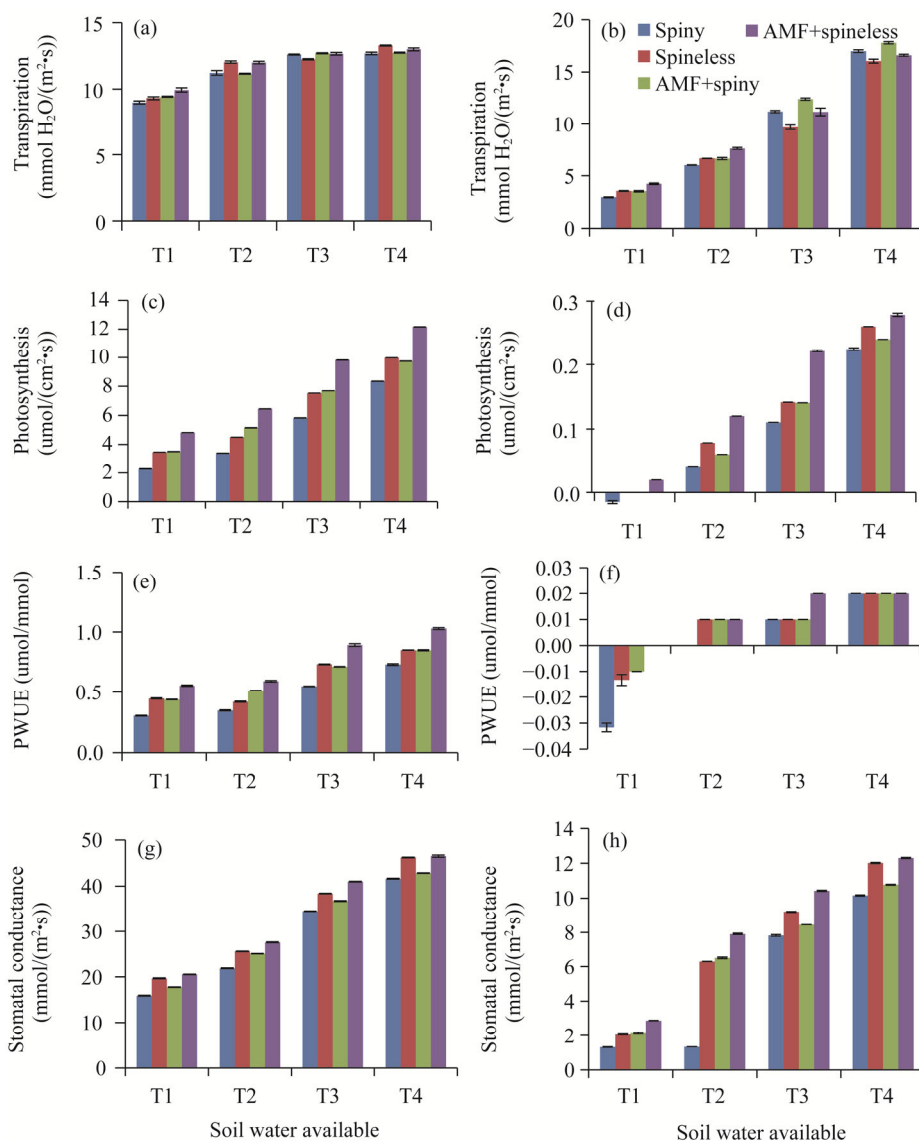

Fig. 3 Effect of interaction of *O. ficus-indica* type (spiny or spineless), arbuscular mycorrhizal fungi (AMF; absent or present), and soil water available (SWA) on morphological traits both during night time (a, c, e, and g) and day time (b, d, f, and h) of *O. ficus-indica* cladodes after 18 months of growth in the greenhouse. PWUE, photosynthetic water use efficiency; T1, 0%–25% SWA; T2, 25%–50% SWA; T3, 50%–75% SWA; T4, 75%–100% SWA. Bars are standard errors.

During night time, mycorrhizal cladodes had shown significantly higher P_n than control cladodes (Table 2). PWUE was significantly higher for mycorrhizal cladodes than non-mycorrhizal cladodes during both day and night times. During day time, PWUE of *O. ficus-indica* plant was observed negative with 0%–25% level of SWA. PWUE was related to P_n of the plant because P_n of *O. ficus-indica* plant can be negative during extreme drought (Nobel and De la Barrera, 1999, 2004).

ANOVA result shows that all physiological traits (P_n, E, C, and PWUE) were significantly related and varied with the time difference (Table 4). Higher physiological traits were observed during night time. It was related to the high water-stress levels of SWA. During day time, the transpiration rate decreases with the increase in surface temperature of the cladodes (Nobel and De la Barrera, 2004). With the water loss of the cladodes, P_n and PWUE also decreased by improving the degree of stomatal closure during drought stress (Nobel and De la Barrera, 1999, 2004; Liguori et al., 2013).

Table 4   Effect of time on physiological traits of *O. ficus-indica* daughter cladodes

Time	Photosynthesis ($\mu mol/(cm^2 \cdot s)$)	Transpiration ($mmol/(H_2O\ m^2 \cdot s)$)	stomatal conductance ($mmol/(m^2 \cdot s)$)	Water use efficiency ($\mu mol/mmol$)
Day	0.123 ± 0.01^b	9.72 ± 0.48^b	7.34 ± 0.33^b	0.01 ± 0.00^b
Night	6.65 ± 0.27^a	11.71 ± 0.14^a	31.72 ± 1.00^a	0.63 ± 0.02^a
F	306.980	167.319	251.990	305.366
P	0.000	0.000	0.000	0.000

Note: Different lowercase letters within day and night times indicate significant differences at $P<0.05$ level. Mean±SD.

5   Conclusions

The potential of *O. ficus-indica* to adapt to drought stress is not only related to its morpho-physiological performance but also its association with AMF. AMF caused an increase in biomass production, increased tolerance to drought stress, and improved photosynthesis and water use efficiency performance of *O. ficus-indica*. Under drought stress, some planted mother cladodes with the absence of AMF have not established daughter cladodes, whereas AMF-inoculated mother cladodes fully established daughter cladodes. High degrees of AMF root colonization were recorded under low plant soil water availabilities. The level of water supply affects the impact of AMF root colonization performance on *O. ficus-indica*. AMF has interacted with decreasing levels of SWA. Thus, mycorrhizal *O. ficus-indica* plants with low levels of SWA increased their benefits. AMF associated with *O. ficus-indica* type and SWA increase growth and improve biomass, P_n, and PWUE. The presence of spine on the surface area of *O. ficus-indica* cladodes can cause decreased growth, biomass, height, breadth, and surface area by reducing P_n and PWUE. These results have direct consequences for the management of AMF technology. Drought stress and dominancy of spiny *O. ficus-indica* plants in the areas should be considered into account to improve the early growth of *O. ficus-indica* and biomass, and allow the rapid establishment of *O. ficus-indica* daughter cladodes.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization: Teame G KEBEDE, Emiru BIRHANE; Methodology: Teame G KEBEDE, Emiru BIRHANE; Formal analysis: Teame G KEBEDE; Writing - original draft preparation: Teame G KEBEDE; Supervision: Emiru BIRHANE, Kiros-Meles AYIMUT, Yemane G EGZIABHER; Writing - review and editing: Teame G KEBED, Emiru BIRHANE, Kiros-Meles AYIMUT, Yemane G EGZIABHER.

References

Aiqun C, Main G, Shuangshuang W, et al. 2017. Transport properties and regulatory roles of nitrogen in arbuscular mycorrhizal symbiosis. New Phytologist, 74: 80–88.

Alho L, Carvalho M, Brito I, et al. 2015. The effect of arbuscular mycorrhiza fungal propagules on the growth of subterranean clover (*Trifolium subterraneum* L.) under Mn toxicity in *ex situ* experiments. Soil Use and Management, 31(2): 337–344.

Andrade J L, Cervera J C, Graham E A. 2009. Microenvironments, water relations, and productivity of CAM plants. In: De la Barrera E, Smith W K. Perspectives in Biophysical Plant Ecophysiology: A Tribute to Park S. Nobel. Mexico: Universidad Nacional Autónoma de México, 95–120.

Andrino A, Guggenberger G, Sauheitl L, et al. 2020. Carbon investment into mobilization of mineral and organic phosphorus by arbuscular mycorrhiza. Journal of Biology and Fertility of Soils, 57: 47–64.

Astello-Garcia M G, Cervantes I, Nair V, et al. 2015. Chemical composition and phenolic compounds profile of cladodes from *Opuntia* spp. cultivars with different domestication gradient. Journal of Food Composition and Analysis, 43: 119–130.

Auge R M, Toler H D, Saxton A M. 2016. Mycorrhizal stimulation of leaf gas exchange in relation to root colonization, shoot size, leaf phosphorus and nitrogen: A quantitative analysis of the literature using meta-regression. Frontiers in Plant Science, 7: 1084, doi: 10.3389/fpls.2016.01084.

Belay T, Gebreselassie M, Abadi T. 2011. Description of cactus pear (*Opuntia ficus-indica* (L.) Mill) cultivars from Tigray, northern Ethiopia. In: Research Report No. 1. Tigray Agricultural Research Institute, Mekelle, Tigray, Ethiopia.

Berhe Y K, Portillo L, Vigueras A L. 2022. Resistance of *Opuntia ficus-indica* cv 'Rojo Pelon' to *Dactylopius coccus* (Hemiptera: Dactylopiidae) under greenhouse condition. Journal of the Professional Association for Cactus Development, 24: 290–306.

Birhane E, Sterck J F, Fetene M, et al. 2012. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Journal of Physiological Ecology, 169: 895–904.

Birhane E, Gebremedihin K M, Tadesse T, et al. 2017. Exclosures restored the density and root colonization of arbuscular mycorrhizal fungi in Tigray, northern Ethiopia. Ecological Processes, 6: 33, doi: 10.1184/s13717-017-0101-9.

Birhane E, Gebretsadik K F, Taye G, et al. 2020. Effects of forest composition and disturbance on arbuscular mycorrhizae spore density, arbuscular mycorrhizae root colonization and soil carbon stocks in a dry afromontane forest in northern Ethiopia. Diversity, 12(4): 133, doi: 10.3390/d12040133.

Brundrett M, Bougher N, Dell B, et al. 1996. Working with mycorrhizas in forestry and agriculture. AClAR Monograph, 32: 374.

Chen X B, Zhu D Q, Zhao C C, et al. 2019. Community composition and diversity of fungi in soils under different types of *Pinus koraiensis* forests. Acta Petrologica Sinica, 56(5): 1221–1234.

Cristina C, Alessandro R, Olubukola O B, et al. 2017. Soil: Do not disturb, mycorrhiza in action. In: Varma A, Prasad R, Tuteja N. Mycorrhiza-Function, Diversity, State of the Art (4th ed.). Berlin: Springer, 27–38.

Frew A, Powell J R, Hiltpold I, et al. 2017. Host plant colonisation by arbuscular mycorrhizal fungi stimulates immune function whereas high root silicon concentrations diminish growth in a soil-dwelling herbivore. Journal of Soil Biology and Biochemistry, 112: 117–126.

Garcia K, Chasman D, Roy S, et al. 2017. Physiological responses and gene co expression network of mycorrhizal roots under K^+ deprivation. Journal of Plant Physiology, 173(3): 1811–1823.

Gou Q Q, Ma G L, Qi J J, et al. 2023. Diversity of soil bacteria and fungi communities in artificial forests of the sandy-hilly region of Northwest China. Journal of Arid Land, 15(1): 109–126.

Hailemariam H, Birhane E, Gebresamuel G, et al. 2017 Arbuscular mycorrhiza effects on *Faidherbia albida* (Del.) A. Chev. growth under varying soil water and phosphorus levels in Northern Ethiopia. Journal of Agroforestry Systems, 92(2): 485–498.

Hu Y, Xie W, Chen B. 2020. Arbuscular mycorrhiza improved drought tolerance of maize seedlings by altering photosystem II efficiency and the levels of key metabolites. Chemical and Biological Technologies in Agriculture, 7: 20, doi: 10.1186/s40538-020-00186-4.

Liguori G, Inglese G, Pernice F, et al. 2013. CO_2 uptake of *Opuntia ficus-indica* (L.) Mill. whole trees and single cladodes, in

relation to plant water status and cladode at Italian. Journal of Agronomy, 8: 14–20.

Loik M E. 2008. The effect of cactus spines on light interception and photosystem II for three sympatric species of *Opuntia* from the Mojave Desert. Physiologia Plantarum, 134(1): 87–98.

Mohammadi M H S, Etemadi N, Arab M M, et al. 2017. Molecular and physiological responses of Iranian perennial ryegrass as affected by trinexapac ethyl, paclobutrazol and abscisic acid under drought stress. Plant Physiology and Biochemistry, 111(1): 129–143.

Nobel P S, De la Barrera E. 1999. Carbon and water balances for young fruits of platyopuntias. Physiologia Plantarum, 109(2): 160–166.

Nobel P S, De la Barrera E. 2004. CO_2 uptake by the cultivated *Hemiepiphytic cactus*, *Hylocereus undatus*. Annals of Applied Biology, 144(1): 1–8.

Nobel P S. 2010. Desert Wisdom, Agaves and Cacti, CO_2, Water, Climate Change. New York: iUniverse, 198.

Ochoa M J, Barbera G. 2017. History, economic and agro-ecological importance. In: Inglese P. Crop Ecology, Cultivation and Uses of Cactus Pear. Rome: Food and Agriculture Organization of the United Nations, 13–19.

Parniske M. 2008. *Arbuscular mycorrhiza*: The mother of plant root endosymbioses. Nature Reviews Microbiology, 6(10): 763–775.

Pena-Valdivia C B, Luna-Cavazos M, Carranza-Sabas J A, et al. 2007. Morphological characterization of *Opuntia* spp: A multivariate analysis. Journal of the Professional Association for Cactus Development, 10: 1–16.

Pereira S, Santos M, Leal I, et al. 2021. Arbuscular mycorrhizal inoculation increases drought tolerance and survival of *Cenostigma microphyllum* seedlings in a seasonally dry tropical forest. Forest Ecology and Management, 492: 119213, doi: 10.1016/j.foreco.2021.119213.

Pimienta-Barrios E, del Castillo-Aranda M E G, Nobel P S. 2001. Ecophysiology of a wild platyopuntia exposed to prolonged drought. Environmental and Experimental Botany, 47(1): 77–86.

Pimienta-Barrios E, Zañudo-Hernàndez J, Nobel P S. 2005. Effects of young cladodes on the gas exchange of basal cladodes of *Opuntia ficus-indica* (Cactaceae) under wet and dry conditions. International Journal of Plant Sciences, 166(6): 961–968.

Ranjan P, Ranjan J K, Misra R L, et al. 2016. Cacti: Notes on their uses and potential for climate change mitigation. Genetic Resources and Crop Evolution, 63: 901–917.

Salem-Fnayou A B, Zemni H, Nefzaoui A, et al. 2014. Micromorphology of cactus-pear (*Opintia ficus-indica* (L.) Mill.) cladodes based on scanning microscopies. Micron, 56: 68–72.

Scalisi A, Morandi B, Inglese P, et al. 2015. Cladode growth dynamics in *Opuntia ficus-indica* under drought. Environmental and Experimental Botany, 122: 158–167.

Snyman H A. 2004. Effect of various water application strategies on root development of *Opuntia ficus-indica* and *O. robusta* under greenhouse growth conditions. Journal of the Professional Association for Cactus Development, 34: 35–61.

Snyman H A. 2005. A greenhouse study on root dynamics of prickly pears, *Opuntia ficus-indica* and *O. robusta*. Journal of Arid Environments, 65(4): 529–542.

Snyman H A. 2013. Growth rate and water use efficiency of cactus pears *Opuntia ficus-indica* and *O. robusta*. Journal Arid Land Research and Management, 27(4): 337–348.

Stevenson A, Hallsworth J E. 2014. Water and temperature relations of soil Actinobacteria. Environmental Microbiology Report, 6(6): 744–755.

Stevens B M, Propster J R, Öpik M, et al. 2020. Arbuscular mycorrhizal fungi in roots and soil respond differently to biotic and abiotic factors in the Serengeti. Mycorrhiza, 30: 79–95.

Taghizadeh M H, Farzam M, Nabati J. 2023. Rhizobacteria facilitate physiological and biochemical drought tolerance of *Halimodendron halodendron* (Pall.) Voss. Journal of Arid Land, 15(2): 205–217.

Tiznado-Hernández M E, Fortiz-Hernández J, Ojeda-Contreras Á J, et al. 2010. Use of the elliptical mathematical formula to estimate the surface area of cladodes in four varieties of *Opuntia ficus-indica*. Journal of the Professional Association for Cactus Development, 12: 98–109.

Trejo D, Barois I, Sangabriel-Conde W. 2016. Disturbance and land use effect on functional diversity of the arbuscular mycorrhizal fungi. Journal of Agroforest System, 90(2): 265–279.

Wang D D, Zhao W, Reyila M, et al. 2022. Diversity of microbial communities of *Pinus sylvestris* var. *mongolica* at spatial scale. Microorganisms, 10(2): 371, doi: 10.3390/microorganisms10020371.